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CELLULOSE - FROM SOLID WASTE TO CHEMICAL RESOURCE

by

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INTRODUCTION

Virtually all of the solar energy captured by the earth, past and present, has been entrapped through the mechanism of carbohydrate synthesis. Photosynthetic plants and photoplankton fix carbon dioxide into simple sugars and store these as complex polysaccharides. (1) It has been estimated that one such polysaccharide, cellulose, is produced at a rate of one hundred billion tons per year or about 150 lbs of cellulose per day for each of the worlds 3.7 billion people. (2)

As a renewable resource, man has utilized this material to the extent that it comprises approximately half of our municipal and industrial solid waste. Though most such materials are burned, buried, or otherwise discarded, the continuing material and energy shortages have forced man to look seriously at a more complete utilization of our renewable cellulosic resources. The technology for production of food, fuel and industrial chemicals from cellulose is presently being developed and refined at many laboratories in the United States and throughout the world. This paper reports on the sources and availability of waste cellulose and discusses several of the most significant processes being investigated for future utilization of this valuable commodity.

SOURCES OF WASTE CELLULOSE

In the U.S. there are about 600 million tons of collectable waste biomass produced annually which is primarly cellulose (3). These can be broadly classified into the areas of industrial wastes, agricultural wastes and municipal refuses. The primary chemical component of all of these substances is cellulose.

Industrial Wastes

By far the largest amounts of industrial cellulosic wastes are produced by the pulp and paper industry. Vast quantities of sludges, wood residues and other fiberous materials are generated during the various phases of pulp manufacture and papermaking. For the most part, these materials are currently discarded, usually as landfill. Located throughout the country, plant operations are concentrated in New England, the Southeastern states and the Pacific Northwest. Since, to a great extent, centralized collection has already been established, they would make ideal cellulosic substrates for conversion to useful products.

Large quantities of feed lot wastes (manure from ruminents) are also produced throughout the country. When washed, the fiberous residue is primarily cellulose. Most of this material is presently collected and discarded or used as fertilizer.

Agricultural Wastes

Tremendous amounts of cellulosic agricultural wastes are generated throughout the world from a wide variety of sources. As an example, in Central America, harvesting of sugar cane results in an estimated 800,000 tons of bagasse each year. During the collection of corn, rice, wheat and other grains, the stalks and leaves are often left in the fields to be wasted or plowed back under. Processing of these grains and other food crops yields vast quantities of cellulosic by-products and residues such as oat and rice hulls, distillers grains, cotton linters, grape pomaces, and coffee bean pulps. In this country, peanut shells are generated at a rate of 800 million pounds per year. While some of these materials are collected and burned as fuel, their full potential is not being realized. The cultivation of land for production of food is carried out in most countries of the world. The wastes and by-products associated with collection and refining of these crops offer the most widespread and abundant sources of cellulosic materials.

During forest harvesting operations, the stumps, roots, branches, and small trees are usually left behind. With proper modern methods of whole tree harvesting, these parts could become valuable cellulose sources available in large quantity. As more effective methods of utilizing cellulosics are developed, cellulose plantations, where plant species are grown because of their high solar energy conversion efficiencies ("BTU bushes") may become a reality.

Municipal Refuse

Roughly one half of the municipal refuse generated in our towns and cities is cellulosic in nature. The total U.S. trash pile for 1973 was estimated by the Environmental Protection Agency at 130 million tons.

The problem of what to do with the ever increasing mountains of trash is one which continues to plague more and more communities. Most of this material is presently disposed of by incineration or landfill - neither a satisfactory solution. Burning often only converts a solid waste problem to an air pollution problem while already scarce landfill sites are rapidly becoming filled. Solid waste treatment systems like the Black Clawson Plant in Franklin, Ohio are extremely useful in reducing the volume of trash and classifying the material into the various glass, metal, and fiber fractions. These fiber fractions are primarily cellulose and at a large treatment center would be an excellent continuously available substrate for further conversion.

Present and Future Uses

To an extent, cellulosic wastes have been used for years with varying degrees of success. Some of the more notable examples have been in the area of building materials. Wood chips and sawdust, when combined with adhesive resins, have resulted in strong, high quality pressed board. A corn board has also been developed from unused corn stalks. These corn stalks and other agricultural "leftovers" have also been used as animal feed, while sawdust has been tested as a cattle food supplement.

Waste paper can be recycled until limitations occur due to decreased fiber length. Upon shredding, this paper is also commonly used as packing material. Research is being carried out to study the utilization of the organic fraction of municipal refuse as a fuel for power plant boilers. Much of the bagasse now produced is currently burned as fuel at the sugar cane refineries. In fact, direct combustion is the most effective way to recover the maximum amount of stored energy. However, these methods leave much to be desired in that they fail to effectively take advantage of the unique chemical properties of cellulose. With recycling and resource recovery becoming important by-words, utilization of chemical properties without total distruction (production of CO_2 & H_2O) of the cellulose, in a sense closing the recycle loop with a tighter noose, becomes more and more important. Novel approaches include the production of useful fuels, food, and chemical raw materials from cellulosic wastes. Many of these processes have been proposed and are in various stages of development.

Fuel Production

Several pyrolysis methods are being studied which produce either gaseous or liquid fuels. A process developed by Monsanto produces char and fuel gases from a mixture of solid waste and fuel oil. The Pyrotek process heats shredded solid waste producing a mixture of combustible gases. The Garrett Pyrolysis process uses flash pyrolysis to produce an oxygenated fuel oil from solid waste (3). These we can classify as crude or non-specific, yielding products of variable quality and capable of acting on the entire organic fraction of the solid waste.

The large scale production of methanol utilizing the Purox process (4) for production of synthesis gas (CO and H_2) has been proposed by Dr. Thomas Reed of M.I.T. Synthesis gas would then be catalytically converted to methanol which can be blended with gasoline. Experiments conducted by Dr. Reed show improved performance and fuel mileage with methanol/gasoline blends (5). The synthesis gas can also be converted to ammonia or methane, both valuable chemicals presently produced from petroleum. The production of methane from anaerobic digestion of a mixture of solid waste and nitrogen rich sewage is being investigated by Dr. Wise and his collegues of Dynatech Inc. in Cambridge, MA (6).

These latter two processes, one chemical and the other biological, yield more specific products which though proposed as sources of fuel could be used to produce other valuable chemicals presently derived from petroleum.

Food or Protein Production

The term Single Cell Protein or SCP was coined only a few years ago at MIT, but in a short time has created considerable world wide interest. The concept of growing single cell organisms such as yeasts or fungi for sources of protein for either animal or human consumption has intrigued scientists as a possible solution to the food shortages that exists in many parts of the world. It is not a new idea. Germany produced yeast on wood sugars and used the yeast as a protein source for human consumption during World War II. The economics of this process are such that it was applied only during war time when the country was faced with a severe food and protein shortage.

At present, SCP production from a variety of substrates is being investigated. The Pekilo process developed in Finland produces microfungi grown on spent sulfite liquor. British Petroleum (BP) has found it economical to produce SCP on hydrocarbons and was among the first companies to demonstrate its feasibility in plant scale production.

The General Electric Process for production of protein from feed lot wastes tackled one of the nation's messiest problems (7). Cellulolytic organisms, such as the thermophile isolated by G.E., are capable of metabolizing the cellulose. The organism itself, now a source of protein, can be fed back to the cattle. Much of the present sources of cattle feed such as soybeans and grain could then be diverted for human consumption. Because of numerous difficulties and dubious economics, G.E. has recently suspended its efforts in developing this process. However, a small group at the

University of Pennsylvania, headed by Dr. Humphrey, continues to investigate the use of the organism for production of protein.

On a more positive note, a group of researchers at Louisiana State University have developed a process to convert waste sugar cane bagasse into a source of food (8). Bagasse is a cellulose rich material left after the sugar has been extracted from the cane. The food is again single cell protein, in this case a bacterium, Cellulomonas flavigena. The Bechtel Corporation and the LSU Foundation have formed a joint venture for the purpose of bringing this process to a point from which it can be commercialized.

In general, the production of single cell protein by direct growth of organisms on cellulose suffers from two drawbacks. The first is the complex enzyme system required to break down the cellulose. Many organisms lack certain components to successfully attack and convert the more crystalline fractions of cellulose. To overcome this, the substrates usually require a pretreatment to enhance their susceptibility to microbial attack. Secondly, when grown on an insoluble substrate, up to 40% of the protein (including the digestive enzymes) produced by cellulolytic organisms is extracellular, leaving lesser protein in the cells themselves. A good process must make allowances for this fact and economical techniques must be developed to recover this soluble protein.

Cellulosic Saccharification

As previously mentioned, cellulose is, like starch, a polymer of glucose. As can be seen in Figure 1, the conversion of cellulose to its monomer greatly increases its utility. With glucose as the primary product of saccharification, we have a basic raw material for production of a wide variety of products. Conversion of glucose to other chemicals or raw materials has recently shown promise for production of such things as glucose based detergents (9).

Microbial conversion of glucose to single cell protein is another attractive process. The selection of the single cell organism is no longer limited to cellulolytic microbes. Since most organisms grow well on glucose, one may select a microbe for its taste, protein efficiency ratio, amino and nucleic acid content and general acceptibility as a food source. In addition, levels of intracellular protein are much higher and extracellular protein lower for organisms when they are grown on soluble substrates such as glucose. Therefore, overall protein recovery is high.

Of course glucose can also be used as a carbohydrate source for both human and animal food. As a fermentation substrate, it is possible to produce antibiotics, vitamins, enzymes, solvents such as ethanol, butanol, and acetone, and other chemicals (citric and lactic acids, glycerol, etc). Ethanol can be converted to ethylene which is the starting material for a complete family of plastics.

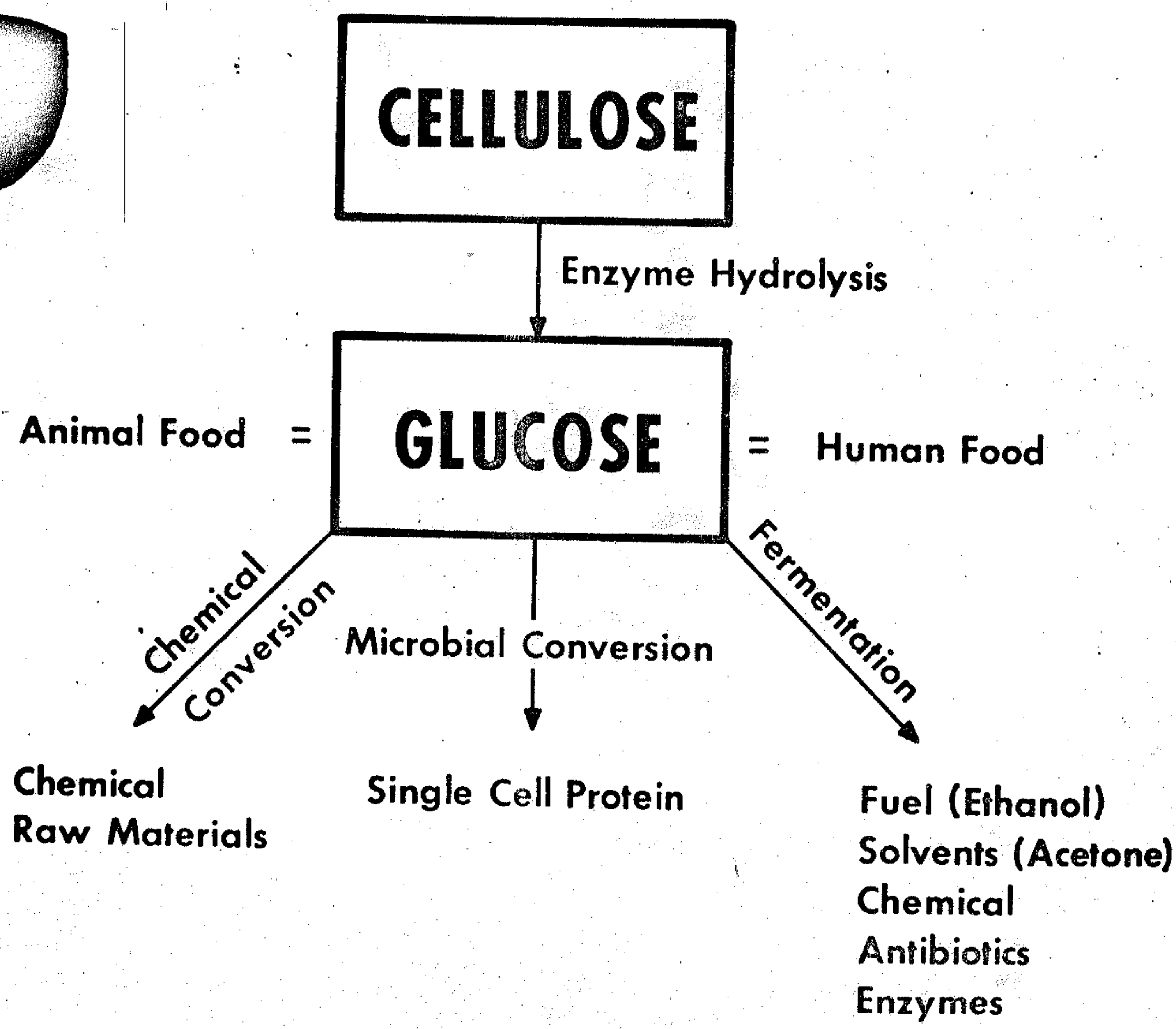

CELLULOSE
Enzyme Hydrolysis
Animal Food =
GLUCOSE
= Human Food
Chemical Conversion
Microbial Conversion
Fermentation
Chemical
Raw Materials
Single Cell Protein
Fuel (Ethanol)
Solvents (Acetone)
Chemical
Antibiotics
Enzymes

Figure 1

The hydrolysis of cellulose to glucose can be carried out acidically or enzymatically. At the Natick Development Center we believe, for several reasons, that enzymatic hydrolysis shows the most promise. First, since acid hydrolysis requires elevated temperatures and pressures with concentrated acids, there is a requirement for very expensive corrosion proof vessels. During World War II, Germany used platinum lined vessels to meet this requirement. Secondly, the acids are not specific in hydrolyzing cellulose and as a result many unwanted compounds are formed which must be removed later in the process.

ENZYMATIC SACCHARIFICATION

Enzymatic conversion, by comparison, is quite mild in its requirements. The reaction takes place at 50 - 55^{0}C, at a pH of 4.8 and since the enzymes are specific, no unwanted reversion compounds are formed.

The process basically duplicates the method used by cellulolytic organisms using the highly specific cellulase enzyme proteins to catalyze the cellulose hydrolysis.

There are many cellulase enzymes involved due to the complex nature of native cellulose and total agreement as to how these enzymes act on the crystalline and amorphous fractions of cellulose has yet to be achieved. In summary, crystalline cellulose must be broken down to a more reactive substrate known as amorphous cellulose which is in turned hydrolyzed through an intermediate product known as cellobiose, the dimer of glucose.

Many organisms consume cellulose and therefore must produce the necessary enzymes for conversion of this substrate. However, there are many organisms which do not produce the enzymes necessary to break down crystalline cellulose. One such example is the LSU bacterium, <u>Cellulomonas flavigena</u>. It is necessary to pretreat the cellulose by swelling the substrate with alkali. This reduces the crystalline fraction and allows the organism to totally utilize the cellulose.

Some organisms are able to totally consume cellulose but do not produce high levels of the cellulase enzyme complex. Such organisms have the enzyme associated with the exterior of the cell wall and produce little free enzyme in solution.

Production of Cellulase Enzymes

The key to any enzymatic process is the production of high quality cell free enzymes. This requires an organism which produces all the necessary enzymes for cellulose degradation, produces them in large quantities and releases them entirely from the cell. One such organism is *Trichoderma viride*. The wild or parent strain has been mutated so that the resulting organism produces approximately four times the quantity of high quality enzyme.

Once the organism was selected and mutated, a process could be developed to produce enzyme and utilize this enzyme to hydrolyze cellulose. Figure 2 shows a proposed processing scheme. Starting with the production of enzyme we see that *T. viride* is grown in submerged culture for a period of approximately four days at which time the mycelium (cell mass) is separated from the broth or "beer" containing the cellulase. This separation is accomplished by a simple filtration. At Natick, the production of high quality enzyme broth has been successfully carried out on a pilot scale. (10).

Hydrolysis of Cellulose

Actual production of sugars takes place by reacting the cellulase with a cellulosic substrate under controlled condition of temperature and pH. There is an ongoing program at NDC for evaluation of various cellulosic

ENZYMATIC CONVERSION OF WASTE CELLULOSE

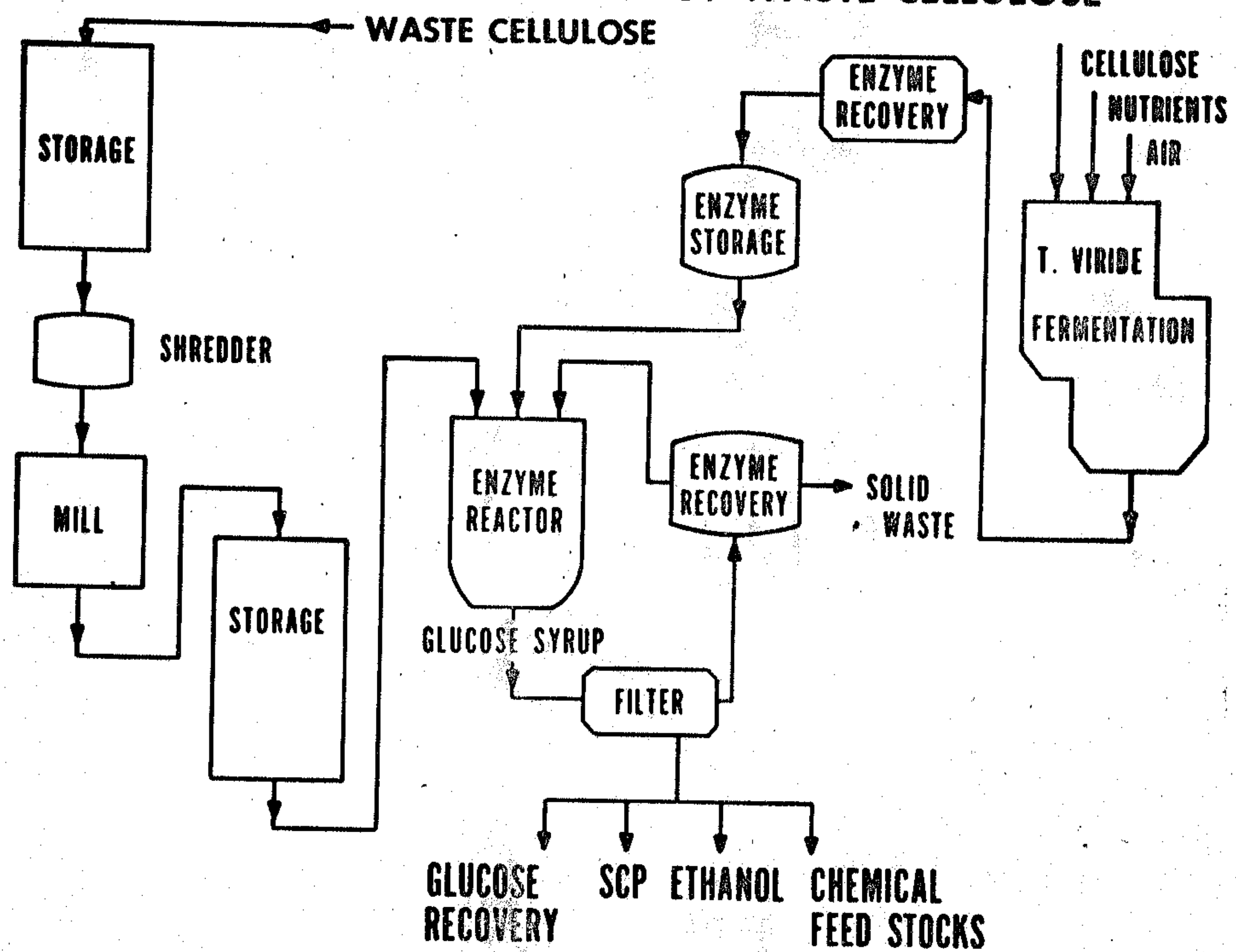


Figure 2

substrates as to their susceptibility to enzymatic hydrolysis. As previously reported (11,12,13), close to one hundred different materials have been tested. These have included paper mill wastes, sludges, and pulps, assorted wood wastes, fiber fractions from municipal trashes treated by Black Clawson, ADL, and Bureau of Mines processors, various grasses, hays, straws, and bagasses, grape pulps, skins, and seeds, newspaper, other waste papers and agricultural processing wastes. These materials are tested in the "as produced" condition and after various milling treatment. A list of representative substrates together with their degree of enzymatic saccharification after 25 hours is shown in Figure 3.

The structure of cellulose as found in nature often carries a high degree of crystallinity. The closely packed crystalline fibrils are very resistant to enzymatic action. To increase the availability, hence the reactivity, of the cellulose a number of physical and chemical methods have been studied (11). Figure 4 shows the effect of various pretreatment on the hydrolysis of newspaper. Of the physical treatments, ball milling is shown to be most effective. This is primarily due to its ability to reduce the crystallinity of the cellulose. Also by increasing the bulk density and reducing the particle size, high suspensions of solids are possible in the reactor.

Currently, hydrolysis is carried out at 50 C and a pH of 4.8. While saccharification readily occurs under these conditions, the actual optimum values for these parameters will be dependent on the type of substrate, the condition of the substrate, and the products desired. Wastes and sludges

INDUSTRIAL WASTES FOR CONVERSION

	SUBSTRATE 5% DRY WT	% SACCHARIFICATION 24 HOURS RELATIVE *		
		AS REC'D WET	AS REC'D DRY	BALL MILLED
	GOOD AS RECEIVED			
22	NICOLET SULFITE PULP	1.2	0.8	1.7
15	HYDROPULPED GOVT. DOCUMENTS	1.3	1.3	1.5
16	HYDROPULPED GOVT. DOCUMENTS	1.1	0.9	1.5
21	NICOLET KRAFT PULP	—	0.8	1.5
12	KIMBERLY CLARK TISSUE MILL WASTE	1.0	1.0	1.1
1	ST. REGIS PAPER MILL SLUDGE	1.0	0.9	0.9
2	ST. REGIS GLASSINE (PVD) WASTE	—	0.8	0.9
3	ST. REGIS GLASSINE (WAX) WASTE	—	0.8	0.6
	GOOD IF BALL MILLED			
13	COTTON LINTERS (MILES)	—	0.2	1.3
18	COREY PAPER MILL WASTE	0.5	0.3	1.2
14	EXTRACTED OAT HULLS (HOFFMAN LA ROCHE)	—	0.1	1.2
20	NICOLET WASTE FILLER	0.6	0.5	1.1
23	RYE GRASS STRAW (MILES)	—	0.3	1.1
17	COVEY PAPER MILL WASTE	0.6	0.5	1.0
26	HERCULES WOOD CHIPS	—	0.1	1.0
25	WELCHES SEEDLESS GRAPE POMACE	—	0.6	0.9
19	STULEY CORN FIBER	—	0.3	0.8
24	WELCHES GRAPE POMACE	—	0.5	0.7

* Ball Milled Newspaper (Ave 42% Sacch) = 1.0

Saccharified at 50° ph 4.8 with _T. Viride_ QM9414
Cellulase 0.08-1.5 w/ml (Ave 1.0)

Figure 3

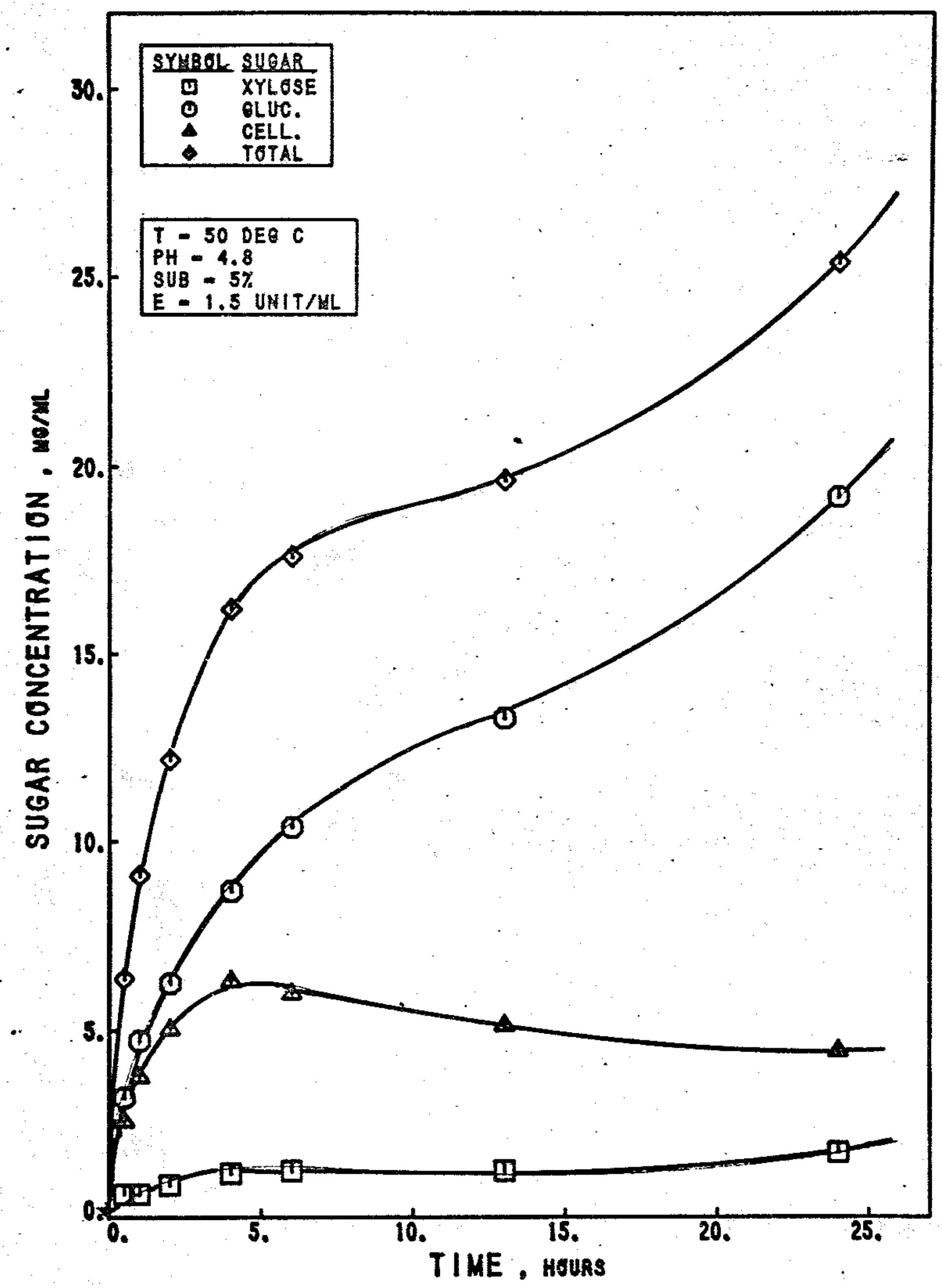
PRETREATMENT OF NEWSPAPER

Newspaper (Boston Globe)	% Saccharification 1 hr	4 hr	24 hr	48 hr
Mighty Mac - Mulcher	10	24	31	42
Jay Bee - Paper Shredder	6	12	24	27
Pot Mill	18	49	65	70
Sweco Mill	16	32	48	56
Granulator-Comminutor	6	14	24	26
Fitzpatrick (Hammer Mill)	10	16	25	28
Majac (Jet Pulverizer)	11	15	26	29
Gaulin (Colloid Mill)	9	17	27	31
Soaked in Water	7	13	24	28
Boiled in Water	4	9	21	26
Treated 2% NaOH	8	14	28	35
Viscose	15	30	44	51
Cuprammonium	18	35	52	58

Figure 4

from paper mills and quality paper products have proven to be most readily saccharified by cellulase enzymes. This is due to both their high cellulose content and to the variety of physical (beating, grinding) and chemical (sulfite or kraft pulping) treatments they undergo during the papermaking process. Many of these materials require no pretreatment, presenting the most favorable economic possibilities. In general agricultural materials require ball milling to break down the crystalline cellulose and make it available for hydrolysis. They also contain less cellulose (40% - 80%). However, they are by far produced in the largest quantities. The fiber fraction of municipal refuse has properties very similar to newspaper. To fully utilize its cellulose content, ball milling would be necessary.

As discussed, the primary component of the hydrolyzate sugars is glucose. Another principal product of saccharification is the dimer cellobiose. This sugar is produced rapidly during the initial reaction phases but decreases in quantity as it is further broken down enzymatically to glucose. This trend is illustrated in Figure 5 for the batch hydrolysis of a glassine paper waste. In this figure, measurable quantities of xylose are also shown to be produced by hydrolysis of the hemicellulose xylan. If the enzymes are produced from Trichoderma viride grown on substrate containing hemicelluloses(primarily xylan and mannan) they will usually contain components capable of hydrolyzing these polysaccharides. The sugars produced (xylose and mannos) can serve as fermentation substrates and xylose can be converted to xylitol or xylonic acid to be used as binders,



COMPOSITION OF SUGARS PRODUCED
DURING HYDROLYSIS OF
GLASSINE-POLYVINYLIDINE WASTE

Figure 5

sweeteners, surfactents or plasticizers.

Lignin

A major consideration in the evaluation of various processes for utilization of cellulosic wastes is the disposition of the noncellulosic portions of the material. The economics will be affected depending upon whether these are considered an additional waste problem or a valuable by product. An advantage of the enzymatic process is that noncellulosic materials pass through virtually unchanged. The most valuable recoverable substance remaining as a residue from the enzymatic hydrolysis of wood products is lignin. Lignin is an amorphous, polymeric substance forming a skeleton within the wood which contributes to the mechanical properties of the cellulose structure and offers a degree of control and protection from environmental effects. It is also an efficient energy storage compound having a heating value of 7,100 cal/gr (14).

The chemical structure of lignin is quite complex as evidenced by the high phenolic content. Various lignins have been subjected to pyrolysis, alkali fusing, hydrogenation, and hydrolysis producing phenol yields as high as 50%. At least 30 different mononuclear phenols produced from lignin have been reported (15). Commercial recovery of the lignin components should become economically viable when methods are developed for utilization of the lignin conversion products other than simple phenols. Breaking down the complex compounds by hydrocracking and reforming the mixtures by catalytic processing, as done in the petroleum industry, would yield simpler more marketable compounds.

CONCLUSIONS

1. There is a rapidly growing awareness by those within the chemical process industry of the potential of cellulosic materials as major sources of food, fuel, and chemicals for the future. As the primary means of harnessing the sun's energy, the photosynthetic process yields tremendous amounts of a valuable renewable organic chemical resource. With diminishing supplies of fossil fuels, strong consideration should now be given to the future replacement of the petroleum economy with one at least partially based on cellulose.

2. There are many sources of cellulosic materials. However, the exact number of different kinds and amounts of each have not as yet been adequately quantified. This is an important part in accurately evaluating the economics of any potential uses of these substrates. The utilization of any noncellulosic parts will heavily affect overall process costs. Further investigation into recovery and production of chemicals from lignin is needed.

3. The enzymatic hydrolysis of cellulose to sugar (primarily glucose) is a feasible method for converting a renewable organic chemical resource to useful products. Glucose can then be used as a microbial substrate to produce a variety of fermentation chemicals, single cell protein, or as a basic chemical feedstock. Work is currently underway to optimize the production of cellulase enzyme and maximize yields of sugar during hydrolysis. Pretreatment of substrate and recovery of enzyme are two major areas requiring further study.

4. We have only touched briefly on the various processes being developed or presently used to exploit our cellulosic resources in nonconventional ways. None of these processes are a panacea; there is probably no single solution; but each offers a potentially economical supplement to either our shrinking oil or food supplies. As the interest in cellulose utilization increases there will undoubtedly be new and alternative processes proposed.

REFERENCES

1. GADEN, E Jr., "Biological Process Systems" A.I.Ch.E. Today Series. Publishe by A.I.Ch.E. New York, New York, 1973.

2. MANDELS, M., NYSTROM, J., SPANO, L.A., Enzymatic Hydrolysis of Cellulosic Wastes. Proceedings of the Fifth Annual Symposium on Environmental Research, Washington D.C., 13 - 14 March 1974.

3. REED, T.B., Proceedings of the Eighth Cellulose Conference, SUNY, Syracuse, New York, 1975.

4. DONGAN, T.A., Proceedings of the Sixth ANERAC Conference, University of Rhode Island, 1975.

5. REED, T., LERNER, R., HINKLEY, E., and FAHEY, R., Inter Society Energy Conversion Engineering Conference, San Francisco, California, 1974.

6. WISE, D.L., SADEK, S.E., Biotech & Bioengr., 1975, In press.

7. TANNENBAUM, S.R., WANG, D.I.C., Single Cell Protein II, MIT Press, Cambridge, Mass., 1975.

8. CALLIHAN, C.D., and DUNLAP, C.E., Report SW-24c, Prepared for Federal Solid Waste Management Program, Louisiana State University, Under Contract # PH86-68-152.

9. Providence Journal - Evening Bulletin, April 1975.

10. NYSTROM, J.M. and KORNUTA, K.A., Proceedings of the International SITRA Symposium on "Enzymatic Hydrolysis of Cellulose" Hammeenlinna Finland, March 1975.

11. MANDELS, M., HONTZ, L. and NYSTROM, J.M., Biotech & Bioengr. 16, 1471 - 93, 1975.

12. ANDREN, R.K., MANDELS, M. and MEDEIROS, J., Proceedings of Eighth Cellulose Conference , SUNY, Syracuse, New York, 1975.

13. ANDREN, R.K., MANDELS, M., and MEDEIROS, J., Proceedings of 170th National ACS Meeting, Chicago, IL, 1975.

14. FALKEHAG, S.I., Proceedings of Eighth Cellulose Conference, SUNY, Syracuse, New York, 1975.

15. GOLDSTEIN, I.S., Proceedings of Eighth Cellulose Conference, SUNY, Syracuse, New York, 1975.